REMARKS

Status of the Claims.

Claims 1-31 are pending with entry of this amendment, claims 32-61 being cancelled and no claims being added herein. Claims 1, 3, 19, and 25 are amended herein. These amendments introduce no new matter. Support is replete throughout the specification and in the claims as originally filed. In particular, as recognized and stated by the Examiner, one of skill in the art would recognize that the blood brain barrier is comprised of cells.

35 U.S.C. §112, Second Paragraph.

Claims 1, 3, and 2, 5-25, 28-31 were rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite because claims 1 and 3 recite "on a cell composing the blood brain barrier." According to the Examiner one of skill cannot determine what is meant by this limitation because the blood brain barrier is comprised of cells and cannot envision the metes and bound of a claim drawn to a single cell that comprises the entire blood brain barrier.

Claims 1, 3, and 19 are amended herein to recite "cells comprising the blood brain barrier". As recognized by the Examiner the blood brain barrier is comprised of a number of cells. Thus, reciting "cells" obviates this rejection.

Claim 1 was rejected under 35 U.S.C. §112, second paragraph, there was allegedly insufficient antecedent basis for the limitation "said composition" in line 6. Claim 1 is amended herein to replace "said composition" with "said imaging reagent" which finds support at line 3 of the same claim thereby obviating this rejection.

35 U.S.C. §103(a).

Claims 1-3, 5-25, and 28-31 were rejected under 35 U.S.C. §103(a) as allegedly obvious in light of Penichet *et al.* (1999) *J. Immunol.,* 163: 4421-4426, or Pardridge *et al.* (1995) *Proc. Natl. Acad. Sci., USA,* 92: 5592-5596, in view of Hnatowich (1999) *J. Nucl. Med.,* 40: 693-703, Kurihara and Pardridge (1999) *Cancer Res.,* 54: 6159-6163, Tavitian (1998) *Nat. Med.,* 4: 467-471, and Zhao (1999) *J. Biol. Chem.*, 274(49): 34893-34902. According to the Examiner, Penichet *et al.* teach a method of *in vivo* systemic administration of an imaging reagent to rats where the imaging reagent is

radiolabeled antisense PNA that hybridizes to the rev mRNA of HIV-1 attached to an anti-transferrin monoclonal antibody linked to the PNA by an avidin/biotin affinity tag. Pardridge *et al.* allegedly discloses *in vivo* systemic administration of a ^{125}I-biotin-PNA/Ox26-SA conjugate. According to the Examiner Penichet et al. and Pardridge *et al.* (1995) fail to teach the method where the imaging reagent comprises a targeting ligand that is an antibody that specifically binds to an insulin receptor, where the nucleic acid is labeled with a radio labeled amino acid that is 111-indium, or wherein the vertebrate is a human.

Kurihara *et al.* is cited as allegedly teaching the directed targeting of an EGF peptide radiopharmaceutical to image brain tumor where delivery is enabled to undergo transport through the blood brain barrier (BBB) because of conjugation of a monoclonal antibody that transcytoses through the BBB and the EGF peptide is labeled with ^{111}In. Hnatowich *et al.* alleged teaching antisense as an imaging tool using radiolabeled DNA. Tavitian *et al.* allegedly teach that antisense oligonucleotides are promising new pharmaceuticals that must be modified to avoid rapid degradation and non-specific binding and to allow membrane passage. Zhao *et al.* allegedly teaches the widespread expression of the insulin receptor mRNA in rat brain cells. Applicants traverse.

The Examiner is respectfully reminded that *prima facie* case of obviousness requires that the combination of the cited art, taken with general knowledge in the field, must provide all of the elements of the claimed invention. When a rejection depends on a combination of prior art references, there must be some teaching, suggestion, or motivation to combine the references. *In re Geiger*, 815 2 USPQ2d 1276, 1278 (Fed. Cir. 1987). Moreover, to support an obviousness rejection, the cited references must additionally **provide a reasonable expectation of success**. *In re Vaeck*, 20 USPQ2d 1438 (Fed. Cir. 1991), *citing In re Dow Chemical Co.,* 5 USPQ2d 1529, 1531 (Fed. Cir. 1988).

The Examiner is further reminded that **one cannot properly rely on inherency to support an obviousness rejection**. As stated by the CCPA (now Court of Appeals of the Federal Circuit);

> The inherency of an advantage and its obviousness are entirely different questions. **That which may be inherent is not necessarily known. Obviousness cannot be predicated on what is unknown**. [emphasis added] *In re Shetty,* 566 F.2d 81, 195 USPQ 753, 757 (CCPA 1977)

In the instant case, the claims are directed to methods of imaging *in vivo* gene expression using a construct comprising a labeled nucleic acid linked to a targeting ligand that binds a receptor on cells comprising the blood brain barrier where the construct crosses the blood brain barrier (BBB) and the cell membrane (CM) to enter a brain cell where the construct hybridizes to second nucleic acid, and the signal produced by the detectable label in the brain cell.

As illustrated schematically in Table 1, the claimed methods involve the construct:

1) Crossing the blood brain barrier;

2_ Crossing a brain cell membrane;

3) Hybridizing to a target nucleic acid inside a brain cell;

4) Being present inside the cell in sufficient concentration to provide a detectable signal.

The combined art offers no reasonable expectation of success that the construct recited in the present claims could cross the BBB, cross the CM, bind a target nucleic acid, and be detectable.

The Examiner improperly argues that Penichet *et al.* and Pardridge *et al.* disclose constructs that **inherently** perform the claimed method. Thus, for example, the Examiner states:

> Therefore, Penichet *et al.* do teach a construct that crosses the a [sp] cell membrane and the will appear in the cytosol of a brain cell because the transferrin antibody conjugated to the antisense nucleic acid of Penichet *et al.* was, absent evidence to the contrary, **inherently transcytosed across the cell membrane of the BBB by the transferrin receptor expressed at the BBB and endocytosed into a brain cell by the transferrin receptor expressed at the BCM**. [emphasis added] (Office Action, page 15, lines 11-16)

As explained below, the transport of the construct across both the BBB and CM was not known. **Obviousness cannot be predicated on what is unknown**.

Penichet *et al.*, for example simply discloses the administration of a composition comprising [^{125}I]biotin-PNA bound to anti-TfR *IgG3-C_H3-Av* to a rat. The PNA component is a PNA specific for the *rev* gene of HIV-1. **However, there is no HIV-1 *rev* target in the mouse brain**. Consequently, **the construct disclosed by Penichet *et al.* has no target with which to hybridize**.

While Penichet *et al.* alleges that the construct crosses the blood brain barrier (BBB), this reference **does not** teach that the construct crosses a cell membrane or that the construct appears in

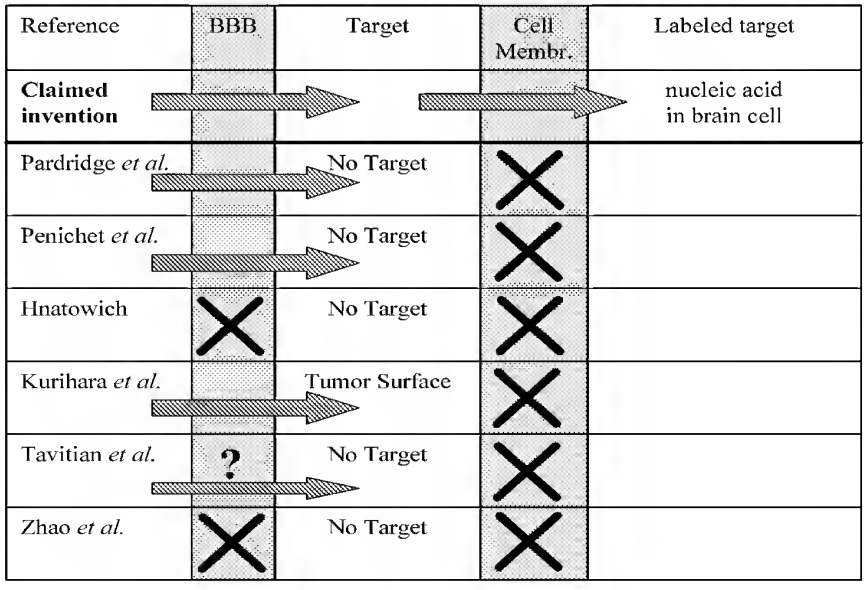
the cytosol of a brain cell in sufficient quantity to permit detection of gene expression. Indeed, Penichet *et al.* **do not** even attempt detection of the PNA construct inside a brain cell. To the contrary, Penichet *et al.* state:

> The plasma and **brain samples were solubilized** with Soluene-350 (Packard Instrument, Saehan, Korea) and neutralized with glacial acetic acid before liquid scintillation counting. The other peripheral tissues, such as liver, kidney, lung, and heart, were also removed and weighted and their radioactivities were counted. [emphasis added] (page 4423, col. 1)

Since brain tissues were removed and solubilized before scintillation counting any extracellular construct would be included in this measurement. Consequently Penichet *et al.* fails to establish that the construct crosses both the BBB and the cell membrane, and fails to establish that the construct enters the cytosol of a brain cell in sufficient quantity to permit detection of gene expression..

Moreover, by teaching extraction and solubilization of brain tissue to detect the labeled construct, Penichet *et al.* effectively **teaches away** from a method of detecting *in vivo* gene expression as recited in the presently pending claim.

Table 1. Schematic illustration of the references and claimed invention.



The Examiner also improperly relies on Pardridge *et al.* as **inherently teaching a construct that crosses the BBB and appears in the cytosol of a brain cell:**

> . . . because the OX26 mAb conjugated to the antisense nucleic acid of Pardridge et al. was, absent evidence to the contrary, **inherently** transcytosed across the cell membrane of the BBB by the transferrin receptor expressed at the BB and endocytosed into a brain cell by the transferrin receptor expressed at the BCM. [emphasis added] (Office action, page 17, lines 4-8)

The transport of the construct across both the BBB and CM was not known. **Obviousness cannot be predicated on what is unknown**. Pardridge *et al.* discloses administration of essentially the same construct as Penichet *et al.* (an anti *HIV-1 rev* PNA coupled to the OX26-streptavidin conjugate) to a rat. This reference simply demonstrates that the construct crosses the blood brain barrier. No evidence is presented to show that the construct also crosses a cell membrane or that is does so in sufficient concentration to permit detection of gene expression. Indeed, this reference merely states:

> Our results are consistent with the following conclusions. (*i*) Free PNAs have very low rates of clearance by brain and other organs (except kidney) and are largely excreted into the urine within 60 min after an intravenous injection. (*ii*) Binding of bio-PNA to the OPX26-SA vector redirects PNA delivery from kidney to organs with abundant transferring receptors, such as liver of the BBB. (*iii*) The bio-PNA bound to OX26-SA is metabolically stable, as shown by measurement of serum TCA-precipitable radioactivity (Fig. 2 Right) and serum HPLC analysis (FIg. 1B). (*iv*) Conjugation of bio-PNA to OX26-SA does not interfere with the PNA hybridization to target mRNA (Fig. 4).

No mention is made regarding uptake into brain cells of the construct. No mention is made of hybridization to a gene transcript **within brain cells**. Indeed, the only measure of the ability of PNA to hybridize to a target was performed *in vitro* using an RNase protection assay.

The combination of Penichet *et al.* and Pardridge *et al.* thus fails to offer a reasonable expectation of success (*i.e.,* that a construct such as that recited in the pending claims could **cross the BBB**, **cross a brain cell membrane**, **hybridize to a target** nucleic acid in **sufficient quantity** to produce a detectable signal).

The remaining cited references, Kurihara and Pardridge, Hnatowich, Tavitian *et al.,* and Zhao *et al.* fail to remedy these defects. Thus Kurihara and Pardridge, for example, disclose the use of an anti-TfR-EGF construct where the EGF is radiolabeled with ^{111}In. Both components of the construct are proteins. Kurihara and Pardridge thus cannot offer a reasonable expectation of success that a nucleic acid construct can cross the BBB, cross the cell membrane, hybridize to a target nucleic acid, and be detectable *in vivo.*

Hnatowich discusses the use of labeled nucleic acids for imaging applications. This reference, however, **does not** disclose the use of labeled nucleic acids attached to a targeting ligand as recited in the presently pending claims. Moreover, Hnatowich expressly teaches that *in vivo* imaging with labeled nucleic acids is problematic (even where passage through the BBB is not required). Thus, Hnatowich expressly states:

> To achieve therapy or **imaging**, antisense DNAs **must cross the cell membrane and enter the cytoplasm without encapsulation and permanent entrapment in endosomal or lysosomal vesicles**. Generally, only **a small percentage** of DNAs incubated with cells are incorporated under the most favorable circumstances (36,37).
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> * * *
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> The **inefficient intracellular localization** of antisense phosphorothioate DNAs explains, in part, the large dosages (e.g. 0.05 mg/kg/h over 10 D) now being administered to patients in connection with antisense chemotherapy (47).
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> **For imaging the problem of inefficient cellular transport may not be so easily resolved** , since simply increasing the dosage of radiolabeled DNAs could decrease target/nontarget radioactivity ratios should the excess labeled DNAs accumulate in normal tissues or show delayed clearance from target tissues. [emphasis added] (page 696 col. 2, paragraph 1)
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> * * *
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> **It is hoped that the problem of poor cellular transport will soon be resolved**, thereby removing perhaps the biggest hurdle to progress in

> antisense chemotherapy and, especially, to the development of antisense imaging. [emphasis added] (page 696 col. 2, paragraph 3)
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> * * *
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> The purpose of this article was to provide a brief description of antisense chemotherapy and to address the question of whether antisense localization can be applied to nuclear medicine imaging. Clearly, antisense imaging would be an extremely valuable diagnostic tool, since, in theory, almost any tissue or disease state could be selectively imaged. **As this contribution may make clear, however, many improvements in the current state of antisense localization will be needed to reach this nirvana**. [emphasis added] (page 703. col 2, paragraph 2)
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> * * *
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> **Existing methods of radiolabeling DNAs with gamma emitters may possible decrease cell membrane transport, interfere with mRNA binding or show intracellular instabilities leading to prolonged nonspecific retention. Alternative methods of radiolabeling will then be needed.** . [emphasis added] (page 703. col 2, paragraph 3)

Hnatowich thus clearly teaches that antisense imaging is fraught with difficulties that have not yet been overcome. Moreover, Hnatowich provides a litany of difficulties supporting Applicants assertion that the cited art provides **no reasonable expectation of success**.

Tavitian *et al.* also leads one of skill in the art to the conclusion that there is **no reasonable expectation of success** that *in vivo* imaging of gene expression in a brain cell is possible using the constructs described in the present application. Tavitian *et al.* disclose the systemic administration to a baboons of a radiolabeled nucleic acid that has no complementary sequence in mammalian cDNA databases. Moreover, Tavitian *et al.* states:

> During the first 5 min after injection (Fig. 1a-c) the radioactivity was high in the heart liver and kidney, and **low in other organs such as the brain** and muscles. [emphasis added] (page 468, col. 1, paragraph 2).
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> * * *
>
> As ON1 is an "orphan" sequence with no biological target in the baboon**, the present radioactivity biodistributions reflect essentially the non-specific interactions** and the metabolic pathways of [18F]ON1. [emphasis added] (page 468, col. 2, paragraph 2).

* * *

> In spite of their promises, **clinical applications of oligonucleotides are still to be awaited because a number of difficulties limit their use *in vivo***. Improvements to be achieved include **resistance to plasma and tissular nucleases**, **better cell membrane penetration** (a necessary requisite as the target RNA is intracellular), and reduced toxicity and side effects. [emphasis added] (page 470, col. 2, paragraph 2).

* * *

> **Further investigations will tell us** if the present methodology, which will help to evaluate strategies for more effective delivery of antisense oligonucleotides to target tissues, also represents the first step toward **nucleic acid imaging** with PET. [emphasis added] (page 470, col. 2, paragraph 3).

Like Hnatowich, Tavitian *et al.* clearly teaches that effective imaging has not been accomplished and there are numerous difficulties to be overcome. Tavitian *et al.* thus clearly establishes that there is no reasonable expectation of success for the presently claimed method.

Zhao *et al.* similarly fails to offer any reasonable expectation of success. To the contrary, Zhao *et al.* simply teaches that the insulin receptor is present in brain tissue and may be implicated in cognitive functions. This reference offers no teaching whatsoever regarding *in vivo* administration of an imaging construct. To the contrary, this reference teaches the use of a labeled nucleic acid probe to detect insulin receptor protein expression in 12 µm **brain sections**.

Zhao *et al.* simply offers no teaching whatsoever that would offer a reasonable expectation of success (*i.e.,* that a construct such as that recited in the pending claims could **cross the BBB**, **cross a brain cell membrane**, **hybridize to a target** nucleic acid in **sufficient quantity** to produce a detectable signal).

In summary, the Examiner reliance on Penichet *et al.* and Pardridge *et al.* as **inherently** teaching that the recited construct was transcytosed across the cell membrane of the BBB by the transferrin receptor expressed at the BBB and endocytosed into a brain cell by the transferrin receptor expressed at the BCM is improper since the alleged activity **was not known** and **obviousness cannot be predicated on what is unknown**. These references thus offer no reasonable expectation of success of the claimed method. Moreover, this defect was not remedied by the remaining references as none of the references showed or even suggested that a construct such as those recited in the pending claims

could cross the BBB, cross a cell membrane, hybridize to a target nucleic acid, and provide a detectable signal. Accordingly the Examiner has failed to make his *prima facie* case and the rejection under 35 U.S.C. §103(a) should be withdrawn.

In view of the foregoing, Applicants believes all claims now pending in this application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested. Should the Examiner seek to maintain the rejections, Applicants request a telephone interview with the Examiner and the Examiner's supervisor.

If a telephone conference would expedite prosecution of this application, the Examiner is invited to telephone the undersigned at (510) 769-3513.

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Respectfully submitted,

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